THE EFFECT OF FEEDING SYSTEM ON THE EXPRESSION OF GENES RELATEDWITH FATTY ACID METABOLISM IN BICEPS FEMORIS MUSCLE OF SUNIT SHEEP

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Abstract –The effect of feeding system on the expression of LPL, ACC, FAS, FABP4, SCD, CPT1, PPARγ, FADS1, FADS2 and Elove5 genes in Biceps femoris muscle was studied. Significant differences were found in the expression of genes FAS, CPT1, FADS1 and FADS2. Genes related to fatty acid synthesis and extension (FAS, FADS1 and FADS2) are higher in pastured groups. In grazing groups the relative expression of CPT1, related to β-oxidation process, was 0.533 fold higher than 0.116 in concentrated group. FADS2 and FABP4 showed extremely positive correlation(r = 0.851, P <0.01). Consequently, Feeding system is an important way to manipulate fatty acid composition in meat through altering gene expression of enzymes related with fatty acid metabolism.

Key Words –Gene expression, fatty acid metabolism, feeding system, Sunit sheep

I. INTRODUCTION

Today's consumers are more interested in knowing what they are really eating, and consumption demands have changed from quantity to quality. The increasing demand for safe meat products needs to change the feeding system to achieve good animal performance and favourable intramuscular fat composition[1].In China, more and more Sunit lambs are kept indoors and fed concentrates now. But grazing increases amounts of polyunsatured fatty acids (PUFA), conjugated linoleic acid (CLA) and n-3 PUFA in lamb meat which are favourable regarding human dietary guidelines[2,3].

The fatty acid composition plays an important role in meat quality. Animal fat quality is determined by fatty acid composition and numerous studies show that fatty acid profile can be manipulated through feeding system [4,5].Some study suggested that changes in fatty acid profile due to feeding system implicate changes in mRNA level expression of genes related with fatty acid metabolism [6].Gene expression analyses can be used to research how feeding systems affect fatty acid profile and can lead to the detection of key genes implicated in fatty acid metabolism. The effect of feeding system on the expression of Acetyl-coenzyme A carboxylase α enzyme(ACC), Fatty acid synthase enzyme (FAS), Lipoprotein lipase (LPL), Fatty acid binding protein(FABP4), Stearoyl-CoA desaturase(SCD), Carnitine palm-itoyltransferase 1 (CPT1), fatty acid desaturase 1(FADS1), fatty acid desaturase 2 (FADS2), fatty acid elongase5 (Elove5) and peroxisome proliferating activated receptor gamma (PPAR γ) gene in Biceps femoris muscle in lambs of the Sunit sheep was studied.

II. MATERIALS AND METHODS

2.1. Animals and diets

Twenty Sunit sheep were allotted into two feeding groups (pasture/10 versus concentrated/10). The pastured sheep populations lived freely in wild grassland, fed on natural grass. The concentrated sheep lived in a closed captivity environments, and were fed wholly on artificial diet (cornstalk and corn). All sheep were slaughtered at 12 months of age in the abattoir of Bayannur Agriculture and Animal Husbandry for sampling the Biceps femoris muscle. The samples were snap-frozen in liquid nitrogen and then stored at -80°C for total RNA analysis at a later date.

2.2 RNA extraction and RT-polymerase chain reaction (PCR)

Total RNA was isolated from the frozen tissues using trizol reagent according to the protocol of manufacturer (Takara, Dalian).Total RNA was reverse-transcribed in a total volume of 20 μL using the PrimeScript RT reagent Kit with gDNA

Table 1 Primer sequences for real-time PCR assays

Targets genes	Primers forward and reverse	Amplicon (bp)	Accession number
SCD	F-5´GAGTACCGCTGGCACATCAA- 3´ R- 5´CTAAGACGGCAGCCTTGGAT- 3´	103	NM 001009254
LPL	F-5´TCATCGTGGTGGACTGGCT- 3´ R- 5´CATCCGCCATCCAGTTCATA- 3´	111	NM 001009394
FAS	F-5´CCCAGCAGCATTATCCAGTGT- 3´ R-5´ATTCATCCGCCATCCAGTTC- 3´	110	GQ150557.1
ACC	F-5´ATGTTTCGGCAGTCCCTGAT- 3´ R-5´TGTGGACCAGCTGACCTTGA- 3´	110	NM_001009256
FABP4	F-5´AAGAAGTGGGTGTGGGCTTT- 3´ R-5´ATGTTGACCACATCCCCATT- 3´	91	EU301804
CPT1	F-5´TCAACACCACTCGCATCCC- 3´ R-5´CGCCCACTCCACTCTTCC- 3´	116	AJ272435
PPARɣ	F-5´CTTGCTGTGGGGATGTCTC- 3´ R-5´GGTCAGCAGACTCTGGGTTC- 3´	121	AY137204
β-action	F-5´CTCACGGAGCGTGGCTACA- 3´ R-5´GCCATCTCCTGCTCGAAGTC- 3´	107	U39357
FADS1	F-5´CTGCTGTACCTGCTGCACAT - 3´ R-5´ACGGACAGGTGTCCAAAGTC - 3´	161	XM 002699285.1
FADS2	F-5´TGCCAACTGGTGGAACCATCGC - 3´ R-5´GCGGCCCGATCAGGAAGAAGTAC- 3	189	NM 001083444.1
Elove5	F-5´TGCTTCAGTTTGTGCTGACC- 3´ R-5´TGGTCCTTCTGGTGCTCTCT - 3	187	EU747336.2

Eraser (Perfect Real Time) (Takara, Dalian) containing gDNA Eraser and PrimeScript RT Enzyme Mix, and RT Primer Mix. Reactions were incubated for 15 min at 37°C, 5 sec at 85°C, and a final 10 min extension at 4°C. The transcribed cDNA was amplified with TaqDNA polymerase (Takara, Dalian) by polymerase chain reaction (PCR) in a thermo cycler using paired sense and antisense primers (Table 1). Specific primers were designed from GenBank sequences using Primer Premier 5.0.

2.3 Quantitative PCR

The expression levels of all genes were examined by RT-PCR using SYBR Premix Ex Taq (Takara, Dalian).β-Actin was chosen as the internal reference gene. The primer sequences used are shown in Table 1.The reaction (25 ml) contained 2 ml pooled cDNA template, 12.5 ml SYBR Premix Ex Taq (Takara), 2ml forward and reverse primers (1ml each), and 8.5 ml DNase/RNase free water. The qPCR conditions were: 50°C /2 min, 95 °C /10 min, 38 cycles of 95 °C /15 sec and 60 °C /1 min, followed by amplicon dissociation (95 °C /15 sec, 60 °C /15 sec, 95 °C /15 sec).These raw CT values were analyzed with a modified delta-Ct method using a PCR data analysis program to obtain relative quantification values. We chose β-actin as the internal control gene, because the expression level of β-actin is basically consistent in each tissue of the animal body. For the treated samples, evaluation of $2^{-\Delta\Delta Ct}$ indicates the fold change in gene expression relative to the untreated control [7].

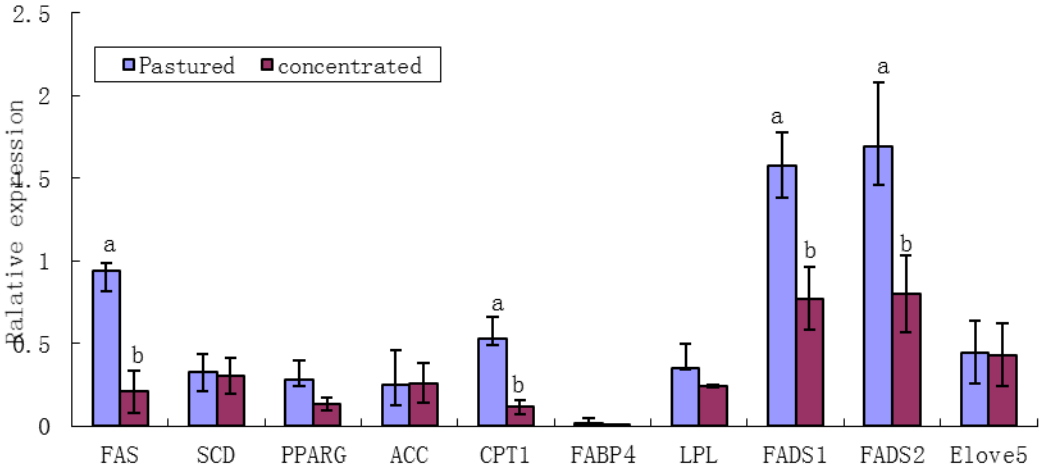
2.4 Statistical analyses

Data were statistically analyzed using SPSS19 for Windows Software. Statistical differences between experimental groups were assessed by one-way ANOVA. Differences were considered statistically significant at $P < 0.05$. Bivariate correlations were used to evaluate the relativity among all genes.

III. RESULTS AND DISCUSSION

All investigated genes in Biceps femoris muscle were expressed in two feeding system. Gene expression was significantly modulated by feeding system. Significant differences in mRNA

Fig.1 Relative gene expression in Biceps femoris muscle of Sunit sheep according to feeding system. Pastured: Sheep grazing ; Concentrated: Sheep in indoor.



expression of 4 genes FAS, FADS1, FADS2, and CPT1 and no Significant differ− rences in mRNA expression of 6 genes SCD, ACC, FABP4, Elove5, PPARγ and LPL were found (Fig. 1).

CPT1 gene expression was higher in pastured group than that in concentrated (P<0.05). The relative expression of the CPT1 gene was 0.533 and 0.116, respectively. Dervishi et al. (2011) showed Grazing lambs have higher levels of the CPT1B gene compared to the indoor groups, as grazing systems promote higher levels of CPT1B gene expression and possibly fatty acid oxidation for energy production. FAS gene catalyzes the synthesis of palmitate from acetyl-CoA and malonyl-CoA, in the presence of NADPH, into long-chain saturated fatty acids [6].The relative expression of the FAS gene was 0.943 and 0.209 fold in pastured group and concentrated groups, respectively. Conversion of ALA into fatty acid metabolites could be influenced by preferential oxidation of this FA or competition for desaturation and elongation enzymes by ALA and LA [8]. The results of study showed that relative expression of the FADS1 gene in pastured group was higher than that in concentrated groups (P<0.05). The FADS2 gene was 1.69 and 0.802 fold in pastured group and concentrated groups, respectively. Several studies in other species have previously showed that dietary n-3 PUFA exert inhibitory effects on genes involved in LCPUFA synthesis [9]. This study suggests that long-term transcriptional regulation to avoid excessive levels of these FA could be one of the mechanisms regulating this type of synthesis, but further research is required to verify this. LPL expression was lower in the concentrated than in the pastured (P>0.05). These results are consistent with those published by Dervishi et al. (2011) in sheep.

The relativity of the ten genes expression in Biceps femoris muscle were analyzed by Bivariate

Table 2 Correlation among all gene mRNA expressing levels in Biceps femoris muscle

	ACC	CPT1	PPARγ	Fabp4	Elove5	LPL	FADS2	FADS1	FAS	SCD
ACC	1									
CPT1	0.333	1								
PPARγ	-0.175	0.049	1							
Fabp4	0.219	-0.541	-0.147	1						
Elove5	0.683	0.506	-0.468	-0.119	1					
LPL	0.017	0.670	-0.035	-0.041	0.075	1				
FADS2	0.783*	0.202	0.248	0.366	0.118	0.173	1			
FADS1	0.618	-0.081	-0.047	0.851**	0.188	0.194	0.720	1		
FAS	0.012	0.063	-0.260	0.552	0.050	0.702	0.111	0.529	1	
SCD	-0.120	0.395	0.102	-0.265	-0.197	0.207	0.024	-0.128	-0.33	1

*:P<0.05; **:P<0.01

Correlations (Table 2). The results showed that PPARγ genes expression were negatively correlated with all gene (except for CPT1 and FADS2) in sheep muscle but the relativity is no significant. PPARγ are members of the nuclear receptor superfamily of ligand-activated transcription factors [10]. PPARγ affects transcription rates of a variety of lipogenic target genes such as FABP, CD36, LPL, Leptin, ACC, FAS, and SCD [11].The two genes (FADS2 and FABP4) showed extremely positive correlation ($r = 0.851$, $P < 0.01$). Furthe-rmore, in Biceps femoris muscle, there was no obvious relationship among other gene ($P > 0.05$).

IV. CONCLUSION

T Due to feeding system , changes in fatty acid profile are affected by changes in expression level of genes related with fatty scid metabolism in Biceps femoris muscle in Sunit sheep. Grazing systems do not only promote higher levels of CPT1 gene expression in semitendinous muscle and fatty acid oxidation for energy production, but also promote the upregulation of genes related to fatty acid synthesis and extension (FAS, FADS1 and FADS2). In the words , the identification of these genes is a good point to start for reference gene selection in SNP detection studies that could be associated with variations in fatty acid composition.

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